

The new claims 127 and 138 are supported by the specification at page 8, third paragraph and page 10, lines 2-13.

The new claims 128-131 and 139-140 are supported by the specification at page 8, the first and third paragraphs; page 10, lines 2-10 and 14-16; and Fig. 6. "Activation" of a polymerase enzyme in Fig. 6 means a release of the polymerase enzyme by reducing the activity of a polymerase-inhibiting agent against the polymerase enzyme. Applicants note that EP 0 771 870, which was incorporated by reference in page 8, the 7th and 8th lines from the bottom of the instant specification, provides support for an inhibitory activity of said polymerase inhibiting agent X and an inhibitory activity of said at least one polymerase-inhibiting agent being "reduced" in claim 129. Page 8, lines 38-39, of EP 0 771 870 discloses that the inhibitory activity of the polymerase-inhibiting agent needs not be totally inactivated to work (EP 0 771 870 is equivalent to Birch, US 5,677,152).

The new claims 132-133 and 141-142 are supported by the specification at page 10, lines 10-13.

The new claims 134-137 are supported by the specification at page 8, first paragraph; the paragraph bridging pages 8 and 9; the paragraph bridging pages 9 and 10; page 10, lines 14-16; and the paragraph bridging pages 10 and 11.

The new claims 143 and 144 are supported by the specification at page 10, the 5th-14th lines from the bottom.

With the addition of claims 127-144, claims 1-144 are pending.

Except for the deletion of "functional derivatives thereof", the amendments to the original claims are made to make the amended claim limitations broader, but no new

matter has been introduced. The new claims 127-144 are added to claim preferred embodiments of the present invention involving the sequential hot-start methods.

Claim Rejections – 35 U.S.C. 112, Second Paragraph

Claims 5-7, 9-12, 41-45, 68-70, 72-75 and 104-108 were rejected as indefinite because of "functional derivatives thereof". Applicants respectfully traverse the rejection. However, to advance prosecution, applicants have deleted such phrase from the claims. Withdrawal of the rejection is requested.

Claim Rejections – 35 U.S.C. 103

Claims 1-126 were rejected as obvious over Koster (US 5,928,906) in view of Gelfand (US 5,310,652) and Birch (US 5,677,152). Applicants respectfully traverse the rejection.

The Office Action asserts that Koster teaches a method for simultaneously amplifying and sequencing nucleic acids by using two different polymerases. Applicants respectfully disagree. As demonstrated with a "Second Declaration under 37 CFR 1.132" filed on March 28, 2000 during the prosecution of the parent application, 08/991,184, the disclosure of Koster is non-enabling because one skilled in the art would have to perform undue experimentation in order to practice the method disclosed by Koster. The "Second Declaration under 37 CFR 1.132" filed on March 28, 2000 demonstrated experimentally that the only working example of Koster was inoperative. Since a method for simultaneously amplifying and sequencing nucleic acids is a very technically challenging proposition, by following merely the general disclosure in Koster,

one skilled in the art would not be able to perform the method for simultaneously amplifying and sequencing nucleic acids without technical guidance provided by the working example, unless the person skilled in the art conducts undue experimentation to find out all the necessary experimental details to make the method work. The only working example of Koster fails to provide any workable technical guidance, so the method was not enabled in Koster. Because a reference must be enabling to be considered prior art, Koster is not proper prior art due to non-enabling disclosure. This is one reason why the obviousness rejection should be withdrawn.

Another reason why the instant claims would not have been obvious over Koster in view of Gelfand and Birch is that Koster does not teach a method using at least one polymerase-inhibiting agent or a method for the simultaneous amplification and sequencing of RNA. Regarding the obviousness rejection of claims 64-99 and 124-126, i.e. the claimed invention related to the use of at least one polymerase-inhibiting agent in a method for simultaneous amplification and sequencing of DNA, the Office Action relies on Birch for the teaching of using a polymerase-inhibiting agent (Abstract and Fig. 1). Applicants respectfully traverse the rejection because Birch merely teaches using the polymerase-inhibiting agent in DNA amplification reactions in PCR reactions (see column 1, lines 10-45) by adding a dicarboxylic acid anhydride to prevent extension of primers non-specifically bound to the template DNA (column 2, lines 59-66). There would have been no reasonable expectation that the polymerase-inhibiting agent of Birch would work in a technically challenging method of simultaneous amplification and sequencing of DNA because one of ordinary skill in the art would not reasonably have predicted that the polymerase-inhibiting agent of Birch would not interfere with the

sequencing reaction that goes on at the same time with the amplification reaction. Manipulations taught by the prior art regarding purely amplification reactions, e.g. PCR, could not be reasonably expected to apply to the technically challenging simultaneous method of amplification and sequencing. Gelfand also fails to provide the reasonable expectation of success required for *prima facie* obviousness. This is another reason why claims 64-99 and 124-126 would not have been obvious over Koster in view of Birch and Gelfand.

Another reason why claims 97 and 114 would not have been obvious over Koster in view of Birch and Gelfand is that claims 79 and 114 require using a polymerase-inhibiting agent **that inhibits the first thermostable DNA polymerase which functions in the claimed method primarily in the sequencing reaction**, and which plays no major role in the amplification reaction. Since Birch teaches adding a polymerase-inhibiting agent **to affect only the amplification reaction**, e.g. in PCR, there would have been no motivation to use Birch's polymerase-inhibiting agent to modify the method of Koster in order to inhibit the first thermostable DNA polymerase, so the method or kit of claim 97 or 114 would not have been obvious.

Another reason why claims 116, 117, 122 and 123 would not have been obvious over Koster in view of Birch and Gelfand is that claims 116, 117, 122 and 123 require an antibody against one of the at least two thermostable DNA polymerases. However, Birch teaches away from using an antibody against a DNA polymerase because Birch teaches that using the antibody has several disadvantages, including being expensive, time-consuming and potentially requiring redesign of the amplification reaction (see

column 2, lines 14-19). Thus, claims 116, 117, 122 and 123 would not have been obvious because one of ordinary skill in the art would not have been motivated by the disadvantages of Birch to add the antibody to the method of Koster.

Other than the non-enablement of Koster, applicants note that the timed-release method ("release" means that the polymerase regains at least a part of its activity after the inhibitory activity of the polymerase-inhibiting agent is reduced) of new claims 134-137 would not have been obvious over Koster in view of Birch and Gelfand because Koster is silent on the timed-release method. Birch and Gelfand provide no teaching of modifying the method of Koster by reversibly reducing the inhibitory activity the polymerase-inhibiting agent at a specific temperature and after a specific number of thermocycles allowing sequencing to start after the DNA has been amplified.

Applicants also note that the timed-release method of the present invention unexpectedly achieved better results as shown in Figure 7.

In addition to the non-enablement of Koster, applicants also note that claims 127-131 would not have been obvious over Koster in view of Birch and Gelfand because Koster does not teach using at least two different polymerase-inhibiting agents against two different DNA polymerases. Birch teaches using only one polymerase-inhibiting agent. Gelfand is totally silent on polymerase-inhibiting agents. This is another reason why claims 127-131 would not have been obvious.

Claims 129 and 130 also would not have been obvious because Koster in view of Birch and Gelfand does not teach or suggest using at least two polymerase-inhibiting agents which are released at different time.

Claims 132, 133, 141 and 142 also would not have been obvious because

Koster, Birch and Gelfand were silent on adding an agent that lowers the melting point of DNA.

Regarding the obviousness rejection of claims 1-36, 59-63 and 37-58, Koster is deficient in not teaching a method to amplify and sequence an RNA in a single container. The Office Action relies on Gelfand for the teaching of a DNA polymerase having reverse transcriptase activity. However, Gelfand teaches the use of the DNA polymerase for only the amplification of RNA. There would have been no reasonable expectation that the teaching of Gelfand would apply to a method for simultaneous amplification and sequencing of RNA. Birch fails to cure the deficiencies of Koster in view of Gelfand. This is another reason why claims 1-36, 59-63 and 37-58 would not have been obvious over Koster in view of Gelfand and Birch.

Withdrawal of the obviousness rejections of claims 1-126 over Koster in view of Gelfand and Birch is requested.

Double Patenting

Claims 1-126 were provisionally rejected as obviousness double patenting over claims 34-58 of 09/357,166 in view of Gelfand and Birch. Applicants respectfully traverse the rejection.

Claims 34-58 of 09/357,166 differ from the instant claims 1-126 in not using at least one polymerase-inhibiting agent or in doing simultaneous amplification and sequencing of RNA. The Office Action attempts to rely on Gelfand and Birch to cure the deficiencies. However, as explained above, Gelfand and Birch fail to cure the deficiencies (e.g. Gelfand and Birch provide no reasonable expectation that modifying

the method of claims 34-58 of 09/357,166 would succeed in simultaneously amplifying and sequencing DNA or RNA; Birch teaches away from using an antibody against the DNA polymerase; and Gelfand and Birch are silent on using two polymerase-inhibiting agents). Withdrawal of the double patent rejection is requested.

Conclusion

With the above reasoning and amendment, applicants respectfully submit that the application is in a condition for allowance.

If this Response is not deemed timely filed, applicants petition for an appropriate extension of time. For the petition fee, if any, and in the event that any fees are due in connection with this paper, please charge our Deposit Account No. 01-2300.

Respectfully submitted,

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Enclosure: Petition for Extension of Time